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Does the Bicoid Gradient Matter?

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The generation and interpretation of positional information are key processes in developmental systems. In this issue, Chen et al. report discoveries made in the *Drosophila* embryo that give new insights into how positional information can be produced by patterning gradients.

One of the most powerful concepts in developmental biology is that of the morphogen. Morphogens are characterized by two general properties: (1) They are distributed in a graded fashion across a tissue or field of cells, and (2) their effects on target cells are concentration dependent. (Rogers and Schier, 2011). The combination of these two properties within a single molecule provides an elegant mechanism for specifying developmental processes as a function of physical distance, that is, for providing spatial information.

The Bicoid (Bcd) protein of *Drosophila* was the first morphogen to be thoroughly described at the molecular level (Driever and Nüsslein-Volhard, 1988). Bcd protein is distributed in a gradient with peak levels at the anterior pole of the syncytial early embryo. The formation of this protein gradient depends on localization of *bcd* mRNA at the anterior tip of the embryo where it provides the source for Bcd protein production and diffusion. An elegant model of how a morphogen can operate in a developmental system is the ''French flag'' concept, which posits that the differential cellular responses elicited by a morphogen are the direct read-out of different threshold levels of the morphogenetic molecule to which a given cell is exposed (Wolpert, 1969). For a long time, the mode of Bcd action appeared to be consistent with a French flag mechanism, implying that Bcd target genes are exquisitely sensitive to changes in Bcd concentration. New work by Chen et al. (2012), challenges this role for Bcd and instead suggests that positional information is specified by a system of repressors that sets the posterior boundaries of anterior Bcd target genes.

The relative simplicity of Bcd gradient formation and its amenability to biophysical, genomic, and computational studies has allowed the characterization of its gradient to an unprecedented degree of accuracy and detail (Porcher and Dostatni, 2010). For the first time the problem of spatial noise in concentration-dependent gene regulation could be addressed systematically (Gregor et al., 2007; Manu et al., 2009). These data demonstrate that a simple read-out of Bcd levels is not sufficient to explain the precise and robust expression borders seen for Bcd target genes expressed centrally in the embryo and that it is rather significant auto- and cross-regulation within the network of target genes in this region that result in the precise specification of positional values (Jaeger et al., 2004; Manu et al., 2009).

The simple model for Bcd morphogenetic action has been dealt another significant blow by the work of Chen et al., appearing in this issue. The authors comprehensively identify and characterize Bcd target genes by using bioinformatic (Bcd site cluster prediction) and biochemical (chromatin immunoprecipitation-on-chip) approaches, combined with validation through reporter gene assays. Building on their results from earlier studies, the authors identified 66 enhancers that direct Bcd-dependent expression. If the corresponding expression domains are aligned according to the position of their posterior borders, they form an almost continuous series covering a region between 20% and 80% of the anterior-posterior axis.

The authors pose the question of whether enhancers that are sensitive to differing levels of Bcd show particular sequence characteristics. A search for the overrepresentation of hexamer sequences uncovers a surprising result: binding sites for the segmentation gene *runt* are significantly more frequent within those enhancers controlling expression in the future head region of the embryo. *runt* is most well known for its later embryonic function in pair-rule patterning, but the authors show that it also has an early expression domain that is graded in the opposite direction to Bcd, and that this early domain is critical for setting the posterior borders of anterior Bcd target genes. The expression domains of these target genes, thus, result from a dual input: activation by Bcd and repression by Runt (Figure 1).

However, the story becomes even more complex, as Runt is not the only repressor involved in Bcd target regulation. The authors also present further evidence that the maternal repressor Capicua and the central gap gene Krüppel (Löhr et al., 2009) are part of the repressor system that sets the posterior boundaries of anterior Bcd target genes. In double-mutant embryos lacking both *runt* and *capicua*, the nesting of head gap gene expression collapses and leads to a failure of head segmentation. Thus, Bcd is not able to exert its proper morphogen function in the absence of the repressor gradients of Runt and Capicua.

The above results show clearly that the Bcd gradient in isolation is not sufficient to robustly set the posterior borders of anterior gene expression but is this gradient necessary? Chen et al. took advantage of a chromosome lacking most activity of the three critical sources of embryonic anterior-posterior polarity, in combination with varying *bcd* copy numbers, to address two main questions: (1) is the steep wild-type Bcd gradient necessary for differential gene expression at the anterior? And (2) are the nuclear levels of Bcd directly correlated with expression of target genes?

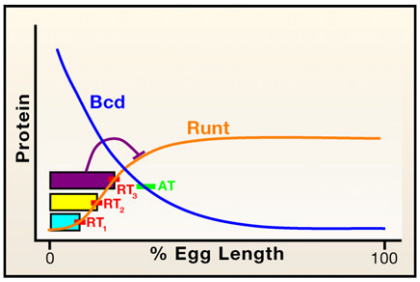

Figure 1. Activator and Repressor Gradients Cooperate for Differential Gene Expression

Anterior target genes (cyan, yellow, and purple bars) integrate activating inputs from Bicoid (Bcd, blue) and repressive inputs from Runt (orange) and Capicua (not shown) in order to establish different posterior borders of expression in fruit fly embryos. Chen et al. (2012) shows that many anterior genes share a common activation threshold (AT) but respond to different repressive thresholds (RT_N). Their work also indicates that the Runt gradient is generated through the action of one or more Bcd target genes (purple line).

The results of these experiments are quite striking. Even flat Bcd gradients may result in sharply defined head gap gene domains that are correctly ordered along the anterior posterior axis. These domains are established at Bcd concentrations that are lower than the corresponding concentrations of the wild-type gradient, suggesting that, in the case of Bcd, the target expression domains do not depend on absolute morphogen levels. This conundrum can be partially explained by the observation that even flat Bcd gradients result in sharp boundaries of *runt* expression and thus in opposing Runt repressor gradients.

The work of Chen et al. shows that neither the wild-type gradient nor the specific levels of Bcd protein are either necessary or sufficient for establishing precise borders of target gene expression at the anterior of the embryo. Given this, is it possible to continue to classify Bcd as a "morphogen"? Based on the criteria of the French flag model, clearly not. However, although the cellular concentration of Bcd protein does not set all thresholds of gene expression in the fly embryo, multiple read-outs of the gradient are detectable. In addition, in the case of *runt*, it appears that the levels and activity of this antagonistic gradient are a function of those of Bcd, indicating that the Bcd gradient is indeed generating most of the positional information in the anterior half of the embryo. Finally, it has been demonstrated in other systems that morphogen interpretation is largely an emergent property of the target gene network (Balaskas et al., 2012).

Thus, by the most general conceptual criteria, Bcd should still be considered a morphogen, just one that is getting even more interesting than we might have imagined.

REFERENCES

Balaskas, N., Ribeiro, A., Panovska, J., Dessaud, E., Sasai, N., Page, K.M., Briscoe, J., and Ribes, V. (2012). Cell *148*, 273–284.

Chen, et al. (2012). Cell *149*, this issue, 618–629.

Driever, W., and Nüsslein-Volhard, C. (1988). Cell *54*, 95–104.

Gregor, T., Tank, D.W., Wieschaus, E.F., and Bialek, W. (2007). Cell *130*, 153–164.

Jaeger, J., Surkova, S., Blagov, M., Janssens, H., Kosman, D., Kozlov, K.N., Manu, Myasnikova, E., Vanario-Alonso, C.E., Samsonova, M., et al. (2004). Nature *430*, 368–371.

Löhr, U., Chung, H.R., Beller, M., and Jäckle, H. (2009). Proc. Natl. Acad. Sci. USA *106*, 21695–21700.

Manu, S., Surkova, S., Spirov, A.V., Gursky, V.V., Janssens, H., Kim, A.R., Radulescu, O., Vanario-Alonso, C.E., Sharp, D.H., Samsonova, M., and Reinitz, J. (2009). PLoS Biol. *7*, e1000049.

Porcher, A., and Dostatni, N. (2010). Curr. Biol. *20*, R249–R254.

Rogers, K.W., and Schier, A.F. (2011). Annu. Rev. Cell Dev. Biol. *27*, 377–407.

Wolpert, L. (1969). J. Theor. Biol. *25*, 1–47.

A piRNA to Remember

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In this issue of *Cell*, Rajasethupathy et al. report a surprising role for piRNAs, previously thought to act mainly in the animal germline to silence transposons, in transcriptional regulation of plasticity-related genes in the central nervous system of the sea slug *Aplysia californica*. The findings expand the functions of small RNAs and have important implications for our understanding of how transient signals can give rise to long-term memories.

During the past decade, small noncoding RNAs have emerged as widely recognized regulators of gene expression and genome stability in eukaryotes ranging from fungi to mammals. Based on their mechanism of biogenesis, small RNAs can be divided into at least two major classes. The first class, which includes miRNAs and siRNAs, is produced from cleavage of double-stranded RNA precursors by the Dicer ribonuclease. The second class, the Piwi-associated small